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Dissection of plant Pol III transcription machinery in *Arabidopsis thaliana*

Our current understanding of RNA polymerase III (Pol III) transcription machinery and its regulation is based mostly on studies in yeasts and vertebrates. In contrast, the knowledge about plant Pol III transcription machinery is very limited. Our long-term goal is to define the proteins and DNA sequences involved in plant tRNA gene expression and elucidate the mechanisms of plant tRNA gene expression and regulation. We have identified Arabidopsis homologs of all subunits of the human Pol III transcription factor TFIIIC: TFIIIC220, TFIIIC110, TFIIIC102, TFIIIC90 and TFIIIC63, and engineered full-length cDNAs to express epitope-tagged proteins in plant suspension cultures and bacteria so as to permit the purification and characterization of the protein-protein interactions between subunits of plant TFIIIC and their binding to tRNA gene. Using a biotinylated tRNALys gene we have shown that the A.tTFIIIC110 subunit binds to the tRNALys gene. Further analysis identified that the DNA-binding domain at its N-terminal half is represented by two A+T–hook motifs first described in HMG-I(Y) protein. Interestingly the Arabidopsis homolog of subunit TFIIIC220, believed to be the main DNA-binding subunit of human TFIIIIC, does not bind to the DNA per se but binds in a complex with AtTFIIIIC110. Preliminary data indicate that the C-terminal end of AtTFIIIC220 interacts with the N-terminal region of AtTFIIIC110. AtTFIIIC90 through its N-terminal half interacts with AtTFIIIC110. Using the system of plasmids from Novagen that allows simultaneous expression in a bacterial host of up to six proteins, we have expressed together all five subunits of Arabidopsis TFIIIC. At present we can detect expression of four subunits with the exception of AtTFIIIC90, and immunopurify a complex containing four subunits. We are working to optimize and improve the expression of all subunits to isolate the AtTFIIIC complex containing all five subunits and study its functional properties in in vitro transcription system.